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Ultrastructural analysis of bone formation in conjunction with the OsteoGen Â®

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ABSTRACT

OsteoGen Â®, has been widely used as material osteoconductor in periodontal surgery. But the structural characteristics of the bone of interphase and neoformated have been poorly evaluated in humans. In order to study the quality of the repair bone around OsteoGen Â®, the material was grafted into bone defects in two patients. Biopsies were taken at 6 and 12 months and processed for optical microscopy (OM) and transmission electron (MET). MO with the results at six months show a clear osteoconduction, which is notorious for the twelve months practically all the particles.

KEY WORDS

Bone, periodontium, biomaterials, graft, osteogenesis, aloplastic

SUMMARY

OsteoGen Â®, has been widely used as osteoconductor material in periodontal surgery. Neverthelees the structural characteristic of the interface and bone neoformated have scarcely assessed in human being. In order to be able to study the quality of the bone repairing around OsteoGen Â®, the material was graft in bone defects in two patients. The biopsies were taken after 6 months and 12 months and processed for optical microscopy (mo) and electronic transmission (met). The results

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KEYWORDS

Bone, periodontium, biomaterial, graft, Osteogenesis, aloplastic.

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INTRODUCTION

OsteoGen Â® (HAResorb Â®, Impladent Ltd., Holliswood, NY.) Is defined as a material osteoconductor, consisting of a synthetic hydroxyapatite slow resorption and no ceramics, and also according to the manufacturer, its crystalline organization is similar to bone human and with no phase containing tricalcium phosphate or pyrophosphates inhibitors of bone mineralization as found in the HA (hydroxyapatite) ceramics. Its manufacturing allows the collection of particles formed by elongated crystals attached to a central core that gives it a large area of contact and hidrofilia by facilitating the absorption of molecules of the extracellular matrix, the repopulation of osteoprogenitoras cells and the subsequent deposition of bone neoformated. With regard to OsteoGen Â®, are scarce research related to its clinical use and histological evaluation especially through biopsies both in human and experimental animals. Ricci et al. histologically verified after twelve weeks of introducing OsteoGen Â®, in a warm dog reabsorption of 80%, a high potential osteoconductivo and chemical properties that prevent the migration of the connective tissue (5). Whittaker et al. taking advantage of autopsy material and human post-mortem as a case report, after six months of testing in the maxillary sinus surgeries with implants osseointegrated, OsteoGen Â®, and DFDBA, found resorption of crystals OsteoGen Â®, its effect osteoconductor, and the effect osteoconductivo the DFDBA (5). The same conclusion Vlassis JM., Et al. in a report of clinical and pathologic case with similar materials (OsteoGen Â®, and DFDBA) used in sinus surgery (6) and Wagner JR. in repairing bone defects after surgery to implant endoseos (7). A. Corsair, a clinical evaluation of 24 bone defects in 16 patients, concluded that having an initial average depth of 4.47 mm probing bone in the reassessment after 4-6 months, there was an average of 2.26 mm new bone , Ie a gain of 51% (8). In conclusion MB Hurzeler et al., In a study of the treatment of Peri-implantitis in Beagle dogs to three months with different modes of therapy concluded that there were no significant differences between the procedures for guided bone regeneration (ROG) and ROG more grafts (Demineralised bone, frozen and dried and OsteoGen Â®), in terms of bone regeneration both from a clinical point of view horn histological (1.2).

Given the importance of using materials aloplastic in bone reconstructive surgery and considering the limited information related to the repair mechanisms OsteoGen Â®, the current study was designed in order to review and assess histologic and ultrastructural level to the biological behavior of OsteoGen Â® , In humans.

MATERIAL AND METHODS

The clinical criteria used to select the two patients involved in this study were as follows: no significant systemic diseases, absence of pharmacological therapy for at least the last six months, to submit an Adult Periodontitis advanced and widespread with at least one or two teeth extractions anterosuperiores indicating reasons for prosthetic, whose average depth clinical probing the outside at least 8 mm., tooth mobility grade 3 and a radiographic bone resorption of 90%. Two patients were chosen that met these requirements, one of 40 years (Caso. 1) and the other 52 years (Caso. 2).

With the assistance and informed consent of patients and after endodontic treatment of the piece chosen, proceeded to make a flap surgery, including coronary odontosecciÃ³n, destartraje supragingival, subgingival, curettage and Radicular polished soft tissue, then were exposed beds surgical intrabony pre-existing defects in which the patient 1 (tooth 8) and Patient 2 (tooth 9) was grafted OsteoGen Â®. Subsequently proceeded to reposition and suturing flaps, for the first intention and protecting the area intervened with surgical cement (GC AMERICA INC CoePack â„¢. USA). Postoperative care included: anti-analgesic therapy based on Ketoralaco, 10 mg every 12 hours., For 3 days (Laboratorio Chile SA) and horn mouthwash chlorhexidine 0.12%, at 12 o'clock., (PerioAid Â®, DENTAID SA , Barcelona, Spain) for two weeks.

The biopsies were obtained after six months (patient 1) and twelve months (patient 2) surgically re-entry, two specimens were removed en bloc, and fixed immediately in a solution of parafomaldehÃdo to 4% to 5% over glutaraldehyde in phosphate buffer , 0.1 Molar pH 7.4 for seven days at 4 Â° C. The samples were hemiseccionadas turbine with high speed and constant cooling using a truncated cone drill, tungsten-carbide, W 557 (SS-WHITE Bursa, Inc, USA). One of the hemisecciones was dehydrated in ethanol upstream and included in LR WHITE Resin HARD GRADE (London Resin Company LTD.) And polymerized at 60 Â° C for 24 hours. The samples were cut using a diamond saw. The other was hemiseccióÃ³n demineralized in 10% EDTA at pH 7.4 for 15 days at 4 Â° C., postfix in osmium tetroxide 1% in phosphate buffer 0.1 Molar pH.-7.4, dehydrated in acetone upstream and included in epoxy resin (Embed 812, EM Sciences). Cuts were made of semi 1micrÃ³n thick that tiÃ±ieron with methylene blue and basic fuchsin and cuts ultrathin 60mm which were mounted on copper grids of 150 mesh. Finally the cuts are ultra tiÃ±ieron citrate with uranyl acetate and lead, and were observed in a transmission electron microscopy (TEM) Zeiss AM-109 operated at 80 kV.

RESULTS

Oplica microscopy (MO)

For the six months (Case 1) we can see that the particles OsteoGen Â®, which surround the implanted tooth roots are colonized by trabeculae of bone tissue spongy gills, while the interface between particles OsteoGen Â®, and the root surface is occupied for a connective tissue fibrillar (figs.1 - 2-3).

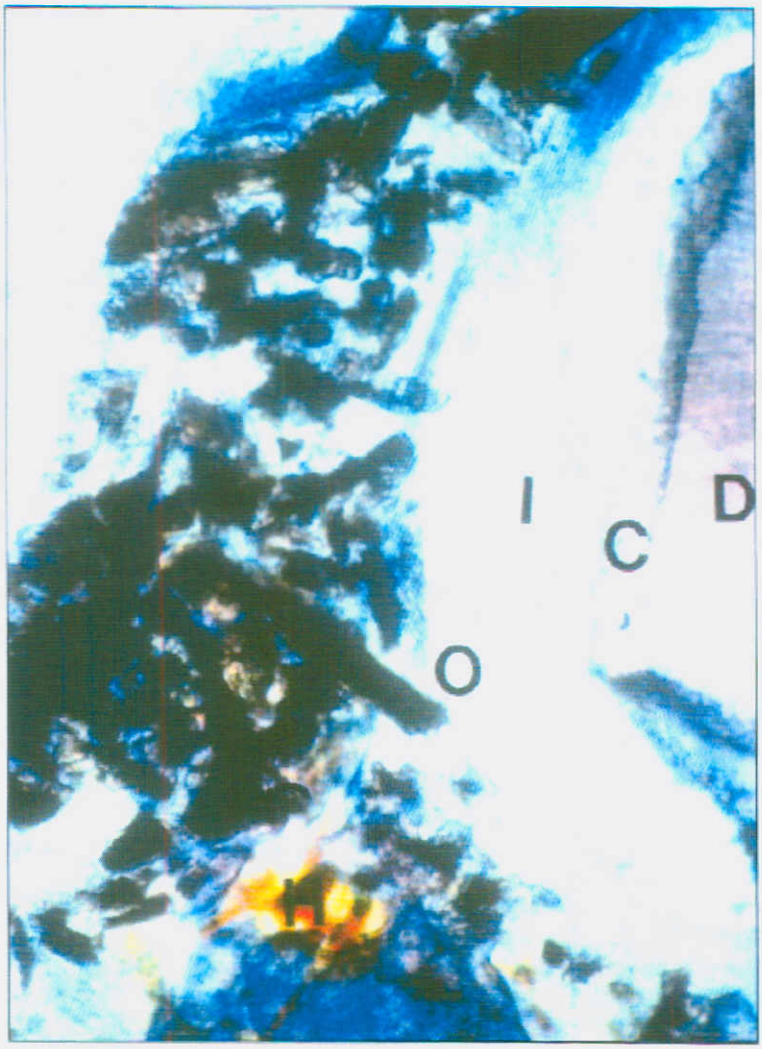

Fig.1: *40x. D: Dentina, C: Cemento, I: Interfase, O: OsteoGen®, H: Hueso.*

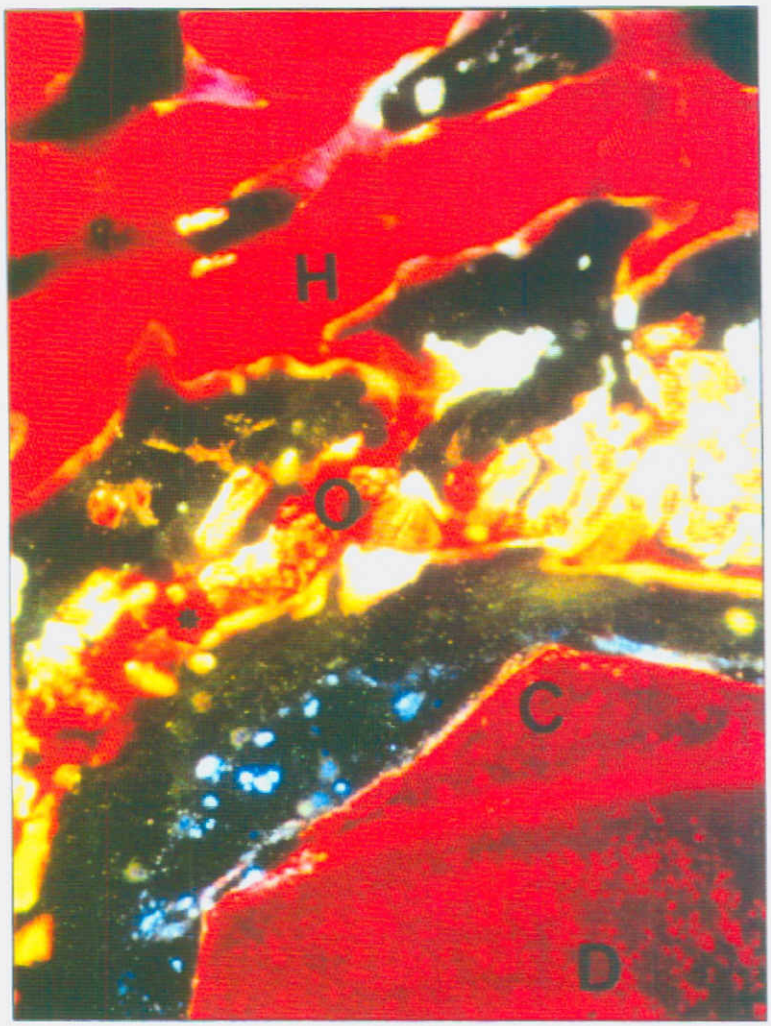

Fig.2: *100x: D: Dentina, C: Cemento, O: OsteoGen®, H: Hueso, *: Trabéculas óseas en contacto directo con las partículas de OsteoGen®.*

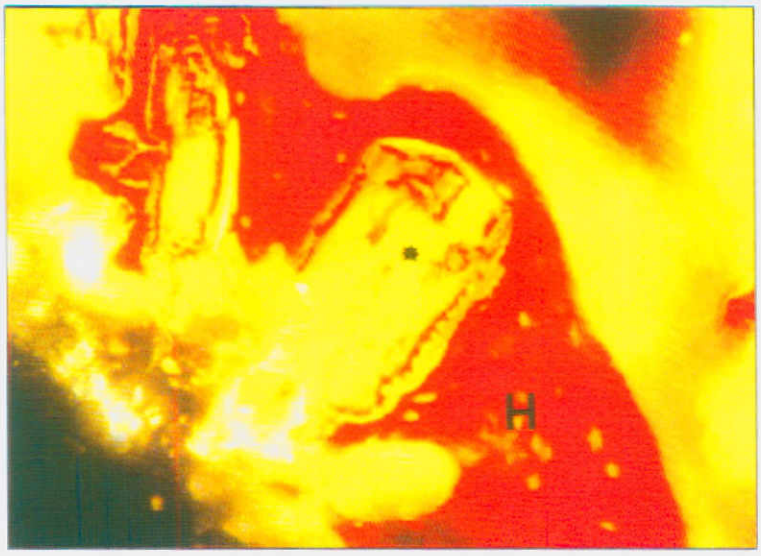

Fig. 3: *400x: O: *, H: Hueso, *: Partículas de OsteoGen®, incluidas en una matriz ósea.*

The areas of the material were not included in the bone matrix presents a different kind of cell populations among which was common to find multinucleated giant cells in intimate contact with the material (Fig. 4).

Fig. 4: *1000x: O: OsteoGen®, H: Hueso, CG: Célula gigante Multinucleada.*

For the twelve months (patient 2) most of the particles are implanted immersed in lamellar bone tissue. (Fig.5)

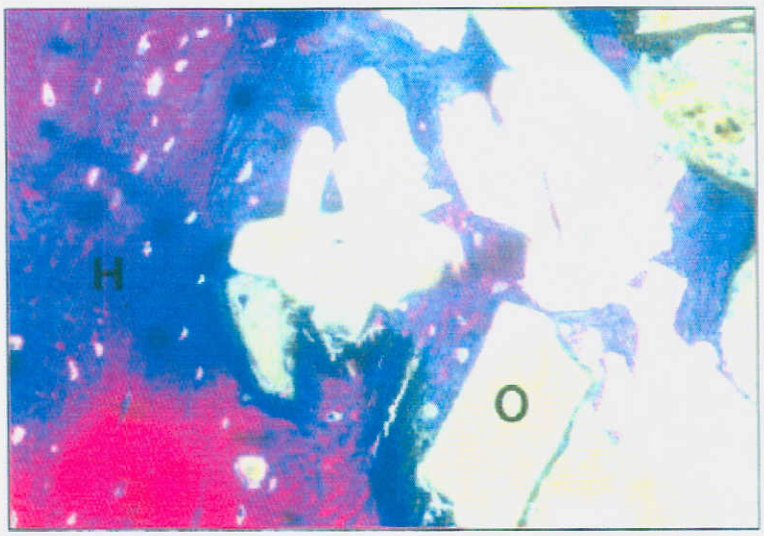

Fig.5: *400x: O: OsteoGen®, H: Hueso.*

Transmission electron microscopy (TEM)

It is observed that the particles are formed by spindle structures, sensitive to EDTA demineralization, surrounded by a matrix electrondensa, finely granular and varying thicknesses. (Fig.6).

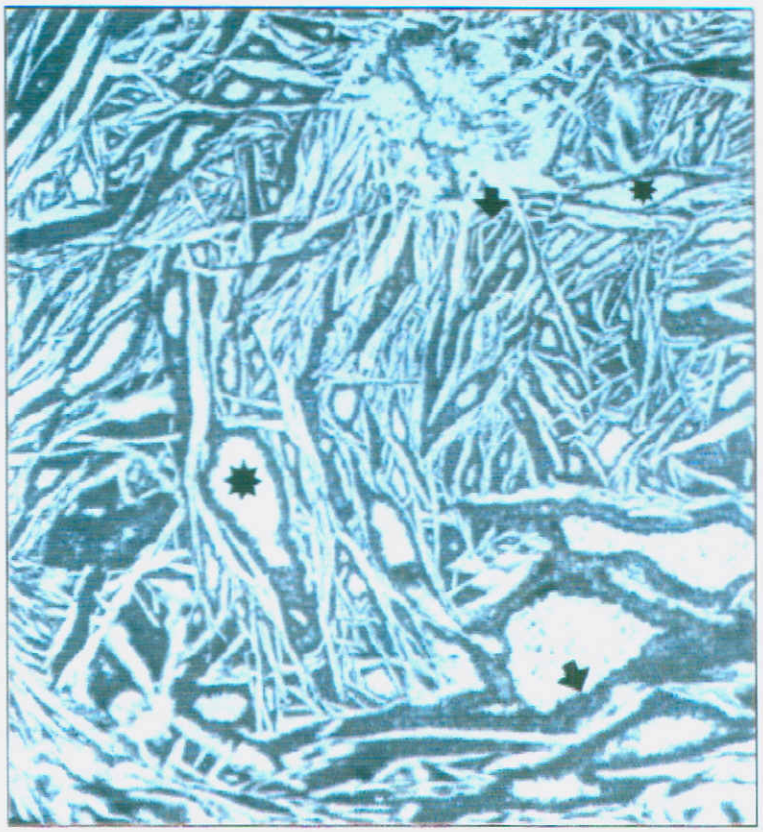

Fig. 6: *Aumento original 7000x, *: OsteoGen®, flecha: Matriz electron-densa granular.*

In both patients shows that the relationship between bone tissue and OsteoGen Â®, can take different forms within a single particle. That is how the interface between the bone and the particle may be held by a wide band of extracellular material, granular-looking filamentous (Fig. 7).

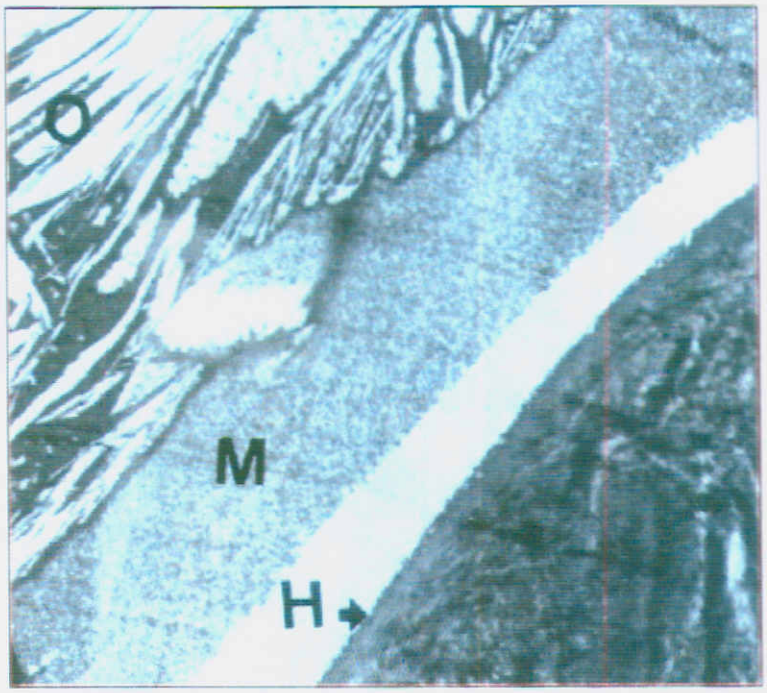

Fig. 7: *Aumento original 4400x, O: OsteoGen®, H: Hueso, M: Material extracelular.*

The transition between the particle and interfacial material is poorly demarcated continuity exists with the material that surrounds the inorganic crystals of OsteoGen Â® (f igs. 8-9).

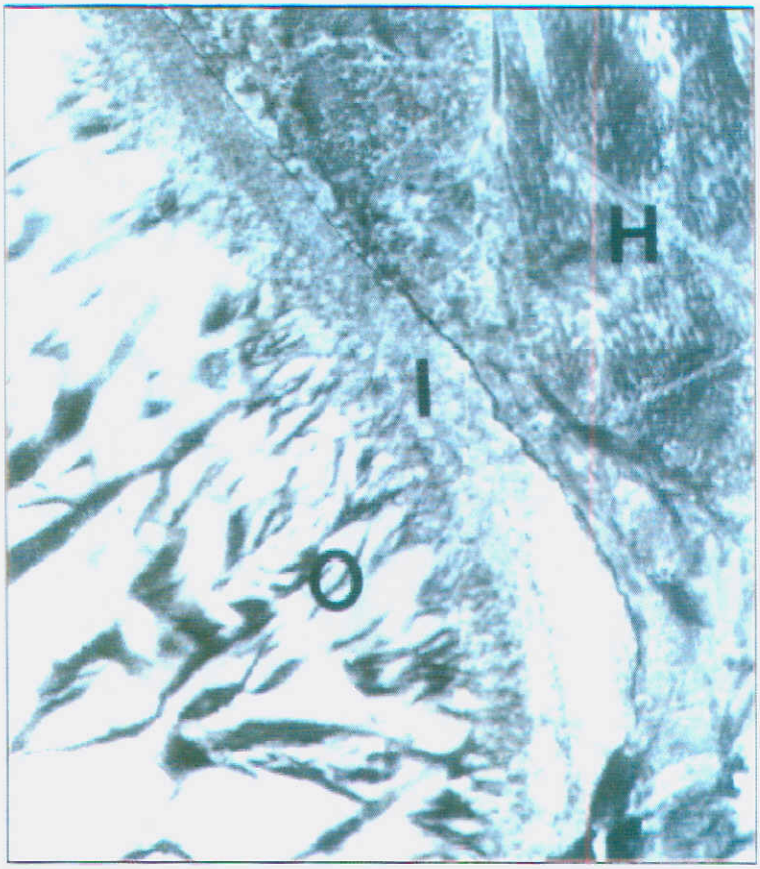

Fig. 8: *Aumento original 7000x, O:OsteoGen®, H: Hueso, I: Interfase.*

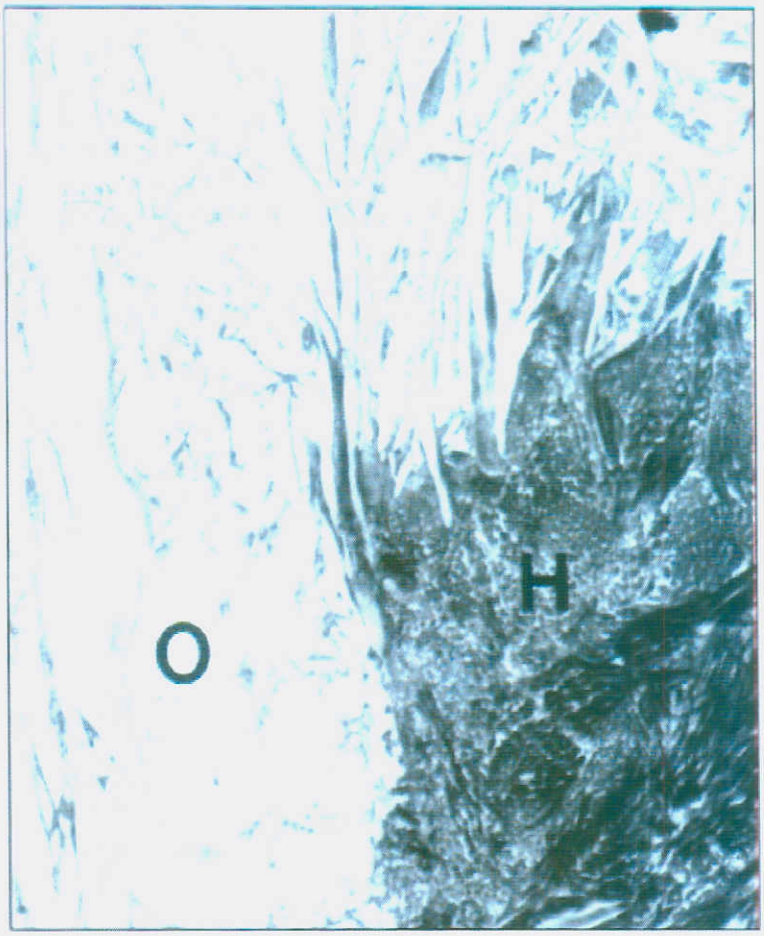

Fig. 10: *7000x: O: OsteoGen®, H: Hueso.*

We detect bone collagen fibrils neoformated invading spaces intercrystalline (figs. 11-12).

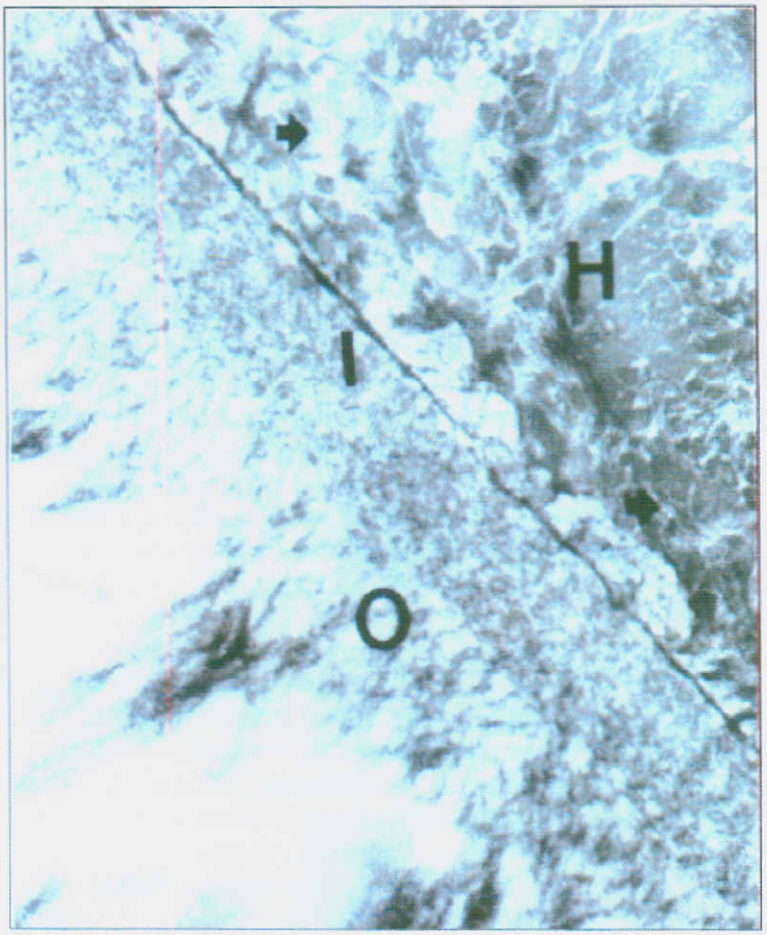

Fig. 9: *Aumento original 200000x, O: OsteoGen®, H: Hueso, I: Material extracelular de la interfase, flecha: Fibrillas colágenas cortadas transversalmente.*

It was further noted that in many areas there is no discernible interface, with intimate contact between the collagen fibrils of bone and the particle (fig.10).

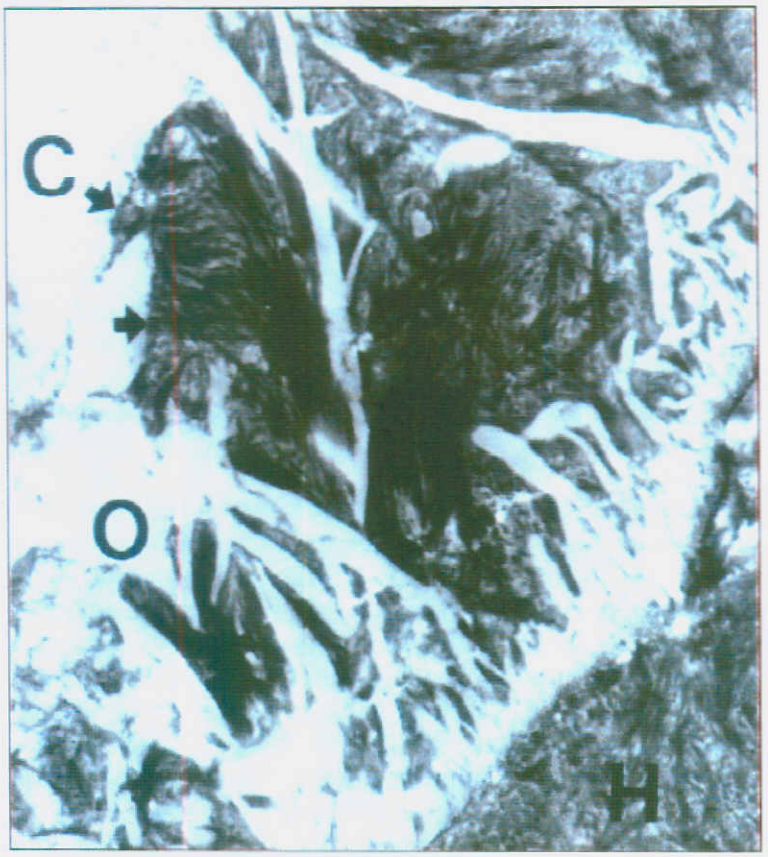

Fig. 11: *7000x: O: OsteoGen®, H: Hueso, C: Fibrillas colagenas en el interior de las partículas de OsteoGen®,(flecha).*

Fig. 12: *Aumento original 20000x, Mayor aumento de la foto anterior en la cual claramente se observan las estriaciones tranversales de la fibrilla de colágeno en el interior de las particulas.*

On the other hand in other areas of the same or other particle interface is occupied by extracellular material electrondenso compact granular appearance and less thick.

DISCUSSION

The osteoconduction, understood to mean that ownership of the materials graft by their physical characteristics that serves as scaffolding around which processes are conducted ossification (9), takes on relevance to the notorious OsteoGen Â®, under experimental conditions of this study and This property is clearly observed in the samples obtained at six months and twelve months. Our observations corroborate the finding of Ricci et al. (4), who noted in Â® OsteoGen to twelve weeks of implanting in warm dog, an intense and osteogenesis osteoconductor. On the other hand, Whittaker et al. Biopsies in OsteoGen Â®, grafted in human maxillary sinus obtained at six months, they noted new bone surrounding the particles (5). With respect to our results, and under these experimental conditions, the particles closest to the root surface showed less bone formation than those that were in direct relation to the walls of the remaining bone defect. This is due, firstly, to the best location of some particles that adapt to anfractuosity bone remnants of walls, allowing the colonization of osteoprogenitoras cells from the bone marrow adjacent, which would be the first to colonize the material to facilitate this so the formation of osteoid tissue. Also, probably some of the material implanted, that is the farthest from the bone, is colonized by non-osteogenesis cells capable of forming a fibrous connective tissue (Connective driving) with no ability to differentiate bone, this is evident especially in the

areas closest to the root surface or connective tissue flap. In this sense, knowing the various factors that hinder or prevent the repair of bone horn flaws in the vascularization, mechanical instability, size of the bone defects and competition in the proliferative activity of tissues participants, it is probably dissimilar to the response of osteoconductiva OsteoGen, (9). For example fibrous connective tissues are proliferating faster than the horn is highly differentiated bone tissue, organizing hÃsticas barriers at the site of the lesion and dramatically slowing the process of bone regeneration. Osteoid tissue can compete with them and finally repaired, but at a rate much lower (9). Therefore it is the importance of all those physical and / or biological processes that promote and support bone regeneration (membranes and / or growth factors).

The nature and functions of the interface cell - biomaterial / bone tissue - biomaterial is controversial. Mazzotti et al. Described it, adjacent to a layer of titanium implants from 30 to 50 microns in width, composed of collagen fibers immature rich glycosaminoglycans in the bone-titanium interface (13), Gross et al. Suggest that the interface of a ceramic vitreous, prior to its calcification located a granular material that was later hipercalcifica (10). Davies et al., Says that on the surface of the implant is deposited Calcitite a substrate, which are inserted the collagen fibers of the extracellular matrix calcified (11). Holtgrave et al., Show a thin band afibrilar interfaces and granular tissue-Osprovit particles, and Interpore-200 (14). In studies of Kenney et al., Only collagen fibers are found traveling the bone tissue newly organized without any layer amorphous interposed with the study material (Interpore-200) (12). Albrektsson et al. Recognize an immediate area to titanium implants composed primarily of proteoglycan and a thickness of 20 to 40 nm (15), however Listgarten et al., Do not identify that area, describing an intimate contact between crystals hydroxyapatite in bone matrix and the surface of titanium without any evidence of a non-mineralized zone (16). In the present study shows that the interface between the OsteoGen Â®, and bone tissue can take different forms, and it is possible to visualize the ultrastructural interface strip granular - amorphous thicknesses of variables. The MET also revealed that the particles in the bone tissue did not present an interface defined, with collagen fibrils of bone matrix in direct contact with them and in the spaces intercrystalline; evidenciarÃan these findings that also involves a osteoconduction microcolonizaciÃ³n of spaces intercrystalline of the particles OsteoGen Â®.

The Proteoglycans taken in the extracellular matrix image ultrastructural associations granular elements and / or filamentary (3). The close structural similarity between the past with the interface OsteoGen Â® - bone detected us to assume that complex protein-polysaccharide (Proteoglycans) mediatizarÃan interaction bone - biomaterial in this study. The use of techniques immunocytochemical corrobar is necessary for this assumption.

The bioreabsorciÃ³n of OsteoGen Â®, a process that is apparently under these experimental conditions is being carried out actively as they observed the presence of mono or multinucleated cells with regard to the material. This has been described as various types of implants are reabsorbed by cells biocerÃ¡micos mono and / or multinucleated. It is well horn Bowell et al. Observe in cell cultures that osteoclasts absorbing surfaces of ceramic materials to create large gaps (17). Klein et al., Says that it is difficult to differentiate between spot a foreign body giant cell and osteoclast, and that only an approximation histoenzimÃ¡tico could be discriminated (18). That is why we refer to phagocytes mono or multinucleated. Osborn et al., Adding that the first cells to adhere to surfaces are ceramic mononuclear phagocytes that preparing for the Osteogenesis (19). In second place in our specimens in the reabsorption occurs smaller excavations which have the same appearance that gaps Howship resulting from the osteoclastic activity described by Nery et al. (20), Hoogendoor et al. (21), Klein et al. (18), and Flattey et al. (22) In this superficial localized resorption, according to Arnett et al. (23), is the process that initiates the formation of mineralized tissues and always precedes bone formation around the grafted material, probably this phase is crucial for a histointegraciÃ³n OsteoGen

Â®.

This fact was not detected in our study because after 6 to 12 months post-implantation, the particles will not be had. Ricci et al. (4), reported in dogs that approximately 80% of the particles OsteoGen Â®, reabsorbe are at 12 weeks. Wagner JR. (7), in a case report in humans, histologically verified the twelve months the presence of phagocytic cells similar to macrophages and / or giant cells on the surface of particles OsteoGen Â®. This is probably due to the reparative process in humans is qualitatively different and much slower than we think. On the other hand Saffar JL et al. (24), states that theoretically everything biomaterial after its implementation can eliminate through a process of dissolution physiological and / or a phenomenon phagocytes dependent. Which of the two predominant has not yet been determined, but if it is clear to the OsteoGen Â®, in this sense that there is a strong cellular response mediated by mono-multinucleated cells.

Recognizing the limitations of this study expressed at the low number of valid samples was further investigated with this or other materials in experimental animals and humans, the clinical and histological properties and corroborate the qualities presented and demonstrated by the manufacturers.

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